Laboratory Toxicity of Three Mosquito Oviposition Repellents to Six Nontarget Aquatic Invertebrates

RUI-DE XUE, DONALD R. BARNARD, AND ARSHAD ALI2

Environ. Entomol. 29(3): 437-441 (2000)

ABSTRACT Toxicity of three mosquito oviposition repellents, N,N-diethyl-3-methylbenzamide (AI3-22542 or deet), AI3-35765, and AI3-37220 to 6 aquatic nontarget invertebrates, was evaluated in the laboratory. The 24-h LC₅₀ values for Cypricercus sp. (Ostracoda), Moina sp. (Cladocera), Eucyclops agilis Koch (Copepoda), Strelkovimermis spiculatus Poinar & Camino (Nematoda), first-and fourth-instar Toxorhynchites amboinensis Doleschall larvae (Diptera), and fourth-instar Chironomus decorus Johannsen larvae (Diptera) ranged from 0.012 to 0.127% or 120 to 1,270 ppm. Cypricercus sp., Moina sp., E agilis, first-instar Tx amboinensis and fourth-instar C decorus were generally more sensitive to the test repellents than male and female S spiculatus and fourth-instar Tx amboinensis. Male S spiculatus was more sensitive to the repellents than its female and this was probably because of the smaller body size of the male. All invertebrates were generally more sensitive to AI3-37220 than to deet and AI3-35765. The experimental repellents for Aedes albopicus (Skuse) mosquitoes.

KEY WORDS Eucyclops agilis, Toxorhynchites amboinensis, Chironomus decorus, Strelkovimermis spiculatus, insect repellents, toxicity

A RECENT LABORATORY and field investigation concerning the insect repellent AI3-22542 or deet (N,Ndiethyl-3-methylbenzamide) and two new experimental mosquito repellents, AI3-37220 and AI3-35765, revealed that the latter two compounds are effective oviposition repellents of Aedes albopictus (Skuse) at rather low rates of application (R.-D.X. and D.R.B., unpublished data). It was also discovered that the two experimental skin repellents, when used as oviposition repellents of container-inhabiting mosquitoes in the laboratory and field, provided sustained mortality of mosquito larvae for several weeks (R.-D.X. and D.R.B., unpublished data). Although the mosquito larvicidal property of these compounds is advantageous for mosquito control purposes, their compatibility with nontarget organisms co-existing with mosquito larvae in the aquatic environments is currently unknown. Among the numerous nontarget benthic invertebrates and zooplankton, species of Ostracoda, Copepoda, Cladocera, Chironomidae, Nematoda, and the predatorial mosquito larvae Toxorhynchites spp. may co-exist with mosquito species in a variety of aquatic ecosystems (Mulla et al. 1979). Consequently, we studied the toxicity of deet, AI3-37220, and AI3-35765 to six selected species of nontarget aquatic invertebrates in the laboratory. This information is essential for understanding of environmental safety of these com-

Materials and Methods

Selection of Test Organisms. Six aquatic invertebrates Cypricercus sp. (Ostracoda), Moina sp. (Cladocera), Eucyclops agilis (Koch) (Copepoda), Strel-kovimermis spiculatus Poinar & Camino (Nematoda), Chironomus decorus Johannsen (Diptera: Chironomidae), and Toxorhynchites amboinensis Doleschall (Diptera: Culicidae) were selected as test organisms. The three crustacean and one chironomid species representing different taxonomic orders and classes were chosen because of their wide distribution and abundance among aquatic communities, their representation from different functional feeding groups, and because of their varying sensitivities to environmental pollution (Dunkel and Richards 1998). The nematode and the predatory mosquito larvae were tested because these

Table 1. Acute toxicity of three mosquito repellents to three different crustaceans, *Cypricercus* sp. (Ostracoda), *Moina* sp. (Cladocera), and *Eucyclops agilis* (Copepoda), collected from the field and exposed to the repellents in the laboratory

			24-h lethal conc, %									
Repellent		Cypricercus sp.			Moina sp.			E. agilis				
		$\overline{\text{LC}_{50}}$	LC_{90}	Slope	$\overline{\text{LC}_{50}}$	LC_{90}	Slope	$\overline{\text{LC}_{50}}$	LC_{90}	Slope		
	AI3-37220	0.012	0.040	2.46	0.012	0.040	2.41	0.015	0.057	2.17		
	AI3-35765											
	AI3-22542	0.012	0.033	2.98	0.024	0.081	2.43	0.014	0.066	1.91		

¹ Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608.

pounds and their field development and use as mosquito oviposition repellents.

 $^{^2}$ University of Florida, IFAS, Mid–Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703-8504.

Table 2. Acute toxicity of three mosquito repellents to a laboratory-reared male and female adult population of the nematode, *Strelkovimermis spiculatus* exposed to the repellents in the laboratory

		24-h lethal conc, %							
Repellent		Male			Female				
	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope			
AI3-37220	0.027	0.077	2.85	0.070	0.437	1.61			
AI3-35765 AI3-22542	$0.036 \\ 0.043$	$0.105 \\ 0.180$	2.79 2.06	0.094 0.127	0.599 0.717	1.59 1.70			

organisms are employed for biological control of mosquitoes in aquatic environments.

Test Repellents. The experimental repellents AI3-37220 [1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine] (98.5% liquid), AI3-35765 [1-(3-cyclohexen-1-ylcarbonyl)-piperidine] (98.5% powder) were provided by Insect Chemical Ecology Research Laboratory, USDA-ARS, Washington, DC. Deet (or AI3-22542, 95% liquid) was purchased from Virginia Chemical, Portsmouth, VA.

Bioassay Procedures. The test animals, mature *E. agilis, Moina* sp., *Cypricerus* sp., and fourth-instar *C. decorus* larvae were collected from outdoor ponds maintained at the USDA's Center for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, FL. Male and female *S. spiculatus* adults tested separately, and first-instar and fourth-instar larvae of *Tx. amboinensis* were obtained from laboratory populations of these organisms maintained at the CMAVE, Gainesville, FL.

For bioassays, 20 individuals of each test organism, except for *Tx. amboinensis* and *C. decorus*, were placed into separate 120-ml disposable plastic cups, each containing 100 ml well water. Five serial dilutions (0.1, 0.05, 0.01, 0.005, and 0.001%) of each compound in acetone were made. For treatments, 1 ml of each concentration (serial dilutions) of a compound was added to a series of five cups; and one cup, maintained as control, received 1 ml of acetone only. Thus, 18 cups were used to test all three compounds simultaneously against one organism. This procedure was also followed for tests with first- and fourth-instar larvae of *Tx. amboinensis* except that only one mosquito larva/cup

Table 3. Acute toxicity of three mosquito repellents to laboratory-reared first- and fourth-instar predatory larvae of the mosquito, *Toxorhynchites amboinensis*, and to field-collected fourth-instar larvae of chironomid midge, *Chironomus decorus*, exposed to the repellents in the laboratory

				24-h le	thal co	onen, %	6			
n 11 .		Tx. amboinensis						C. decorus		
Repellent	1st instar		4th instar			4th instar				
	$\overline{\text{LC}_{50}}$	LC_{90}	Slope	$\overline{\text{LC}_{50}}$	LC_{90}	Slope	$\overline{\text{LC}_{50}}$	LC_{90}	Slope	
AI3-37220	0.017	0.043	3.24	0.056	0.100	5.11	0.017	0.058	2.46	
AI3-35765 AI3-22542										

of an instar was used. Similarly, five field-collected fourth-instar larvae of C. decorus/cup were tested using the procedures of Mulla and Khasawinah (1969). In each test, mortality of a test organism in the cups was scored at 24- and 48-h posttreatment. All three repellents were tested against each species on four different occasions at $24 \pm 1^{\circ}\mathrm{C}$ room temperature and a photoperiod of 14:10 (L:D) h.

Data Analysis. A $3 \times 5 \times 2$ factorial split-plot design (Steel and Torrie 1980) was employed for data analysis of each organism. Factor 1 consisted of three treatment materials (deet, AI3-37220, and AI3-35765), factor 2 was five application concentrations (0.1, 0.05, 0.01, 0.005, and 0.001%) of each material, and factor 3 was two exposure times (24 and 48 h) of each species to the test materials. A computer-based probit analysis (Finney 1971) was used to analyze dosage response of each species to the test materials. For some species, 24-h LC₅₀ and LC₉₀ values were estimated by nonlinear interpolation because of the lack of fit to the linear regression model. A multiway analysis of variance test was separately performed on each species using a computer program (True Epistat Manual 1989); data were transformed using the $\sqrt{x+1}$ transformation to improve homoscedasticity. The mean separation test was not performed because it would not benefit the dose-response toxicity data.

Results

Acute toxicity of the three test oviposition repellents to the ostracod, *Cypricercus* sp., cladoceran

Table 4. Laboratory mortality of field-collected Cypricercus sp. (Ostracoda) exposed to three mosquito repellents in disposable bioassay cups for 24- and 48-h periods at five rates of treatment

_		Repellents ^a and mean \pm SE mortality for 24- and 48-h exposures ^b									
Treatment rate, ^c %	AI3-5	37220	AI3-	35765	AI3-22542 (deet)						
rate, %	24 h	48 h	24 h	48 h	24 h	48 h					
0.1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0					
0.05	100 ± 0	100 ± 0	75 ± 3	100 ± 0	95 ± 2	100 ± 0					
0.01	25 ± 3	35 ± 5	10 ± 2	40 ± 5	45 ± 7	80 ± 3					
0.005	15 ± 1	20 ± 2	10 ± 1	25 ± 4	10 ± 1	25 ± 3					
0.001	3 ± 1	10 ± 3	0 ± 0	7 ± 3	0 ± 0	8 ± 3					
Control	0 ± 0	00 ± 0	0 ± 0	0 ± 0	2 ± 0.4	2 ± 0.4					

Twenty Cypricercus sp./cup; four replicates for each treatment rate and control.

 $^{^{}a}F = 17.27$; df = 2, 10; P < 0.001.

 $^{^{}b}F = 79.07$; df = 1, 10; P < 0.001.

 $^{^{}c}F = 752.72$; df = 5, 10; P < 0.001.

Table 5. Laboratory mortality of field-collected Moina sp. (Cladocera) exposed to three mosquito repellents in disposable bioassay cups for 24- and 48-h periods at five rates of treatment

		Repellents ^a and mean \pm SE mortality for 24- and 48-h exposures ^b									
Treatment rate, ^c %	AI3-	37220	AI3-	35765	AI3-22542 (deet)						
rate, //	24 h	48 h	24 h	48 h	24 h	48 h					
0.1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0					
0.05	100 ± 0	100 ± 0	79 ± 37	100 ± 0	70 ± 37	100 ± 0					
0.01	34 ± 28	98 ± 5	19 ± 17	92 ± 10	20 ± 24	84 ± 15					
0.005	7 ± 11	63 ± 25	3 ± 5	72 ± 4	0 ± 0	44 ± 32					
0.001	7 ± 7	12 ± 8	4 ± 7	10 ± 7	2 ± 2	5 ± 5					
Control	0 ± 0	2 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0					

Twenty Moina sp./cup; four replicates for each treatment rate and control.

Moina sp., and copepod E. agilis is summarized in Table 1. Among the three crustaceans, E. agilis showed a somewhat similar susceptibility response to all three test repellents with an LC_{90} range of 0.057% (AI3-37220) to 0.066% (AI3-22542 or deet). The response of Cypricercus sp. and Moina sp. to AI3-37220 and AI3-35765 was very similar as indicated by their similar LC_{50} and LC_{90} values (Table 1). Among the three compounds tested, deet was the most toxic to Cypricercus sp. ($LC_{90} = 0.033\%$), whereas AI3-37220 was the most toxic to Moina sp. ($LC_{90} = 0.04\%$) as well as to E. agilis ($LC_{90} = 0.057\%$).

Susceptibility data on the nematode S. spiculatus to the three oviposition repellents is shown in Table 2. Based on the LC_{90} values, male S. spiculatus were 3.98–5.7 times more susceptible to the three repellents than the respective females. Among the test repellents, deet was the least toxic and AI3-37220 the most toxic to male as well as female S. spiculatus; the LC_{90} values of male S. spiculatus for all three repellents were generally higher than those observed for the three crustacean invertebrates.

As for the dipterans tested, first-instar larvae of Tx. amboinensis were 2.1–4.2 times more susceptible to the three repellents than the respective fourth instars (Table 3). AI3-37220, AI3-35765, and deet, in that order, were toxic to both larval instars of this predatory mosquito. Against fourth-instar C. decorus midge larvae, AI3-37220 (LC₉₀ = 0.058%) was the most toxic

followed by deet ($LC_{90} = 0.064\%$), and AI3–35765 ($LC_{90} = 0.105\%$), showing that fourth-instar midge larvae were 1.7–3.1 times more susceptible to the three repellents than the fourth-instar larvae of Tx. amboinensis (Table 3). In all tests, the lowest LC_{90} value was that of Cypricercus sp. with deet ($LC_{90} = 0.033\%$) and the highest ($LC_{90} = 0.717\%$) for female S. spiculatus with deet. Thus, up to 21-fold susceptibility difference occurred in the invertebrates tested against the three mosquito oviposition repellents.

Factorial analysis revealed that the test repellents (F = 17.27; df = 2, 10; P < 0.001), treatment rates (F =752.72; df = 5, 10; P < 0.001), and exposure time (F =79.07; df = 1, 10; P < 0.001) affected percent mortality of Cypricercus sp. Also, there was a significant difference between repellents and treatment rate interaction, repellents and exposure time interaction, and treatment rate and exposure time interaction (Table 4). Data in Table 5 show that the repellents (F = 13.48; df = 2, 10; P < 0.001), treatment rates (F = 448.05; df =5, 10; P < 0.001), and exposure time (F = 211.55; df =1, 10; P < 0.001) affected percent mortality of *Moina* sp.; there also was significant interaction between treatment rate and exposure time. In the case of E. agilis, the three repellents did not result in significantly different percent mortalities (F = 3.06; df = 2, 8; P > 0.05); however, the treatment rate (F = 1,043.38; df = 4, 8; P < 0.001), and exposure time (F = 168.75; df = 1, 8; P < 0.001) affected E. agilis mortality (Table

Table 6. Laboratory mortality of field-collected Eucyclops agilis (Copepoda) exposed to three mosquito repellents in disposable bioassay cups for 24- and 48-h periods at five rates of treatment

_		Repellents ^a and mean \pm SE mortality for 24- and 48-h exposures ^b								
Treatment rate, ^c %	AI3-	AI3-37220		35765	AI3-22542 (deet)					
rate, 70	24 h	48 h	24 h	48 h	24 h	48 h				
0.05	100 ± 0	100 ± 0	99 ± 1	100 ± 0	100 ± 0	100 ± 0				
0.01	22 ± 1	95 ± 1	17 ± 1	80 ± 2	22 ± 1	75 ± 2				
0.005	10 ± 0	17 ± 1	9 ± 1	20 ± 1	10 ± 0	12 ± 1				
0.001	2 ± 0	5 ± 1	0 ± 0	4 ± 1	5 ± 0	7 ± 1				
Control	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0				

Twenty E. agilis sp./cup; four replicates for each treatment rate and control.

 $^{^{}a}F = 13.48$; df = 2, 10; P < 0.001.

 $^{^{}b}F = 211.55$; df = 1, 10; P < 0.001.

 $^{^{}c}F = 448.05$; df = 5, 10; P < 0.001.

 $^{^{}a}F = 3.06$; df = 2, 8; P > 0.05.

 $^{^{}b}F = 168.75$; df = 1, 8; P < 0.001.

 $^{^{}c}F = 1043.38$; df = 4, 8; P < 0.001.

Table 7. Mortality of laboratory-reared adult male and adult female parasitic nematode, Strelkovimermis spiculatus, exposed to three mosquito oviposition repellents in disposable bioassay cups for 24- and 48-h periods at five rates of treatment in the laboratory

_	Repellents ^a and mean \pm SE mortality for 24- and 48-h exposures ^b									
Treatment rate, ^c %	AI3-	37220	AI3-3	35765	AI3-22542 (deet)					
rate, 70	24 h	48 h	24 h	48 h	24 h	48 h				
			Adult $male^d$							
0.1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0				
0.05	75 ± 1	95 ± 3	47 ± 4	95 ± 3	28 ± 1	98 ± 9				
0.01	2 ± 1	5 ± 1	8 ± 1	14 ± 1	7 ± 1	14 ± 1				
0.005	2 ± 1	2 ± 1	2 ± 1	4 ± 1	4 ± 1	4 ± 1				
0.001	2 ± 1	2 ± 1	0 ± 0	0 ± 0	2 ± 1	2 ± 1				
Control	2 ± 1	2 ± 1	2 ± 1	2 ± 1	0 ± 0	0 ± 0				
			${\bf Adult\ female}^d$							
0.1	85 ± 2	100 ± 0	70 ± 3	100 ± 0	55 ± 1	95 ± 1				
0.05	15 ± 1	85 ± 3	12 ± 1	77 ± 2	10 ± 1	67 ± 3				
0.01	4 ± 1	9 ± 1	8 ± 1	10 ± 0	3 ± 1	8 ± 1				
0.005	2 ± 1	3 ± 1	0 ± 0	2 ± 1	3 ± 1	3 ± 1				
0.001	4 ± 1	4 ± 1	2 ± 1	2 ± 1	0 ± 0	0 ± 0				
Control	0 ± 0	0 ± 0	2 ± 1	2 ± 1	0 ± 0	0 ± 0				

Twenty adults/cup; four replicates for each treatment rate and control.

6). Also, the repellents and treatment rate, repellents and exposure time, and treatment rate and exposure

time interactions were significant. Table 7 shows that the three repellents did not induce a significantly different mortality of *S. spiculatus*. However, treatment rate (F = 166.32; df = 5, 36; P < 0.001), exposure time (F = 18.77; df = 1, 36; P < 0.001), and sex (F = 14.40; df = 1, 36; P < 0.001) affected percent mortality of this nematode. Also, treatment rate and exposure time, treatment rate and sex, and exposure time and sex interactions were significant.

The test repellents and the exposure times did not have significant difference in percent mortality of larval C. decorus and Tx. amboinensis (Tables 8 and 9). However, treatment rate (F=158.35; df = 5, 10; P<0.001) affected the larval midge mortality and Tx. amboinensis larval mortality (F=19.83; df = 5, 36; P<0.001) as well as larval instar (F=9.13; df = 1, 36; P<0.001). There were no significant differences between any interactions concerning larval C. decorus as well as first- and fourth-instar larvae of Tx. amboinensis.

Discussion

Among the aquatic invertebrates exposed to the various mosquito oviposition repellents in this study, Cypricercus sp. was the most susceptible to deet ($\mathrm{LC}_{50}=0.012\%$ or 120 ppm) and female S. spiculatus was the least susceptible to deet ($\mathrm{LC}_{50}=0.127\%$ or $1,\!270$ ppm). The sensitivity of some test invertebrates differed according to life stage and sex. For example, the first-instar larva of Tx. amboinensis was more sensitive to the test compounds than the fourth instar, and S. spiculatus males were more sensitive than females. This result may be because of the smaller body size of first-instar Tx. amboinensis, compared with the fourth instar; and the same explanation may be true for S. spiculatus males, which are smaller in body size than females.

There are no previous data in the literature concerning the activity of the test repellents against any aquatic nontarget invertebrates for purposes of comparisons. However, the comparatively high LC_{50} val-

Table 8. Laboratory mortality of field-collected Chironomus decorus larvae exposed to three mosquito oviposition repellents in disposable bioassay cups for 24- and 48-h periods at five rates of treatment

		Repellents ^a and mean \pm SE mortality for 24- and 48-h exposures ^b									
Treatment rate, c %	AI3-	37220	AI3-	35765	AI3-22542 (deet)						
rate, %	24 h	48 h	24 h	48 h	24 h	48 h					
0.1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0					
0.05	95 ± 0	100 ± 0	70 ± 2	100 ± 0	80 ± 1	100 ± 0					
0.01	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	10 ± 1					
0.005	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1					
0.001	6 ± 1	6 ± 1	6 ± 1	6 ± 1	0 ± 0	6 ± 1					
Control	0 ± 0	10 ± 1	6 ± 1	6 ± 1	10 ± 1	10 ± 1					

Five larvae/cup; four replicates for each treatment rate and control.

 $^{^{}a}F = 1.20$; df = 2, 36; P > 0.05.

 $^{{}^{}b}F = 18.77$; df = 1, 36; P < 0.001.

 $^{^{}c}F = 166.32$; df = 5, 36; P < 0.001. $^{d}F = 14.40$; df = 1, 36; P < 0.001.

 $^{^{}a}F = 1.24$; df = 2, 10; P > 0.05.

 $^{{}^{}b}F = 3.82$; df = 1, 10; P > 0.05.

 $^{^{}c}F = 158.35$; df = 5, 10; P < 0.001.

Table 9. Mortality of laboratory-reared first- and fourth-instar mosquito larval predator, *Toxorhynchites amboinensis*, exposed to three mosquito oviposition repellents in disposable bioassay cups for 24- and 48-h periods at five rates of treatment in the laboratory

	Repellents ^a and mean ± SE mortality for 24- and 48-h exposures ^b								
Treatment rate, ^c %	AI3-37220		AI3-3	35765	AI3-22542 (deet)				
	24 h	48 h	24 h	48 h	24 h	48 h			
		1st	$instar^d$						
0.1	100	100	100	100	100	100			
0.05	90	100	90	100	60	100			
0.01	30	80	20	70	30	50			
0.005	0	20	0	0	10	20			
0.001	0	20	0	0	0	0			
Control	0	0	0	10	0	10			
		4tł	n instar ^d						
0.1	90	90	60	90	50	60			
0.05	40	50	0	20	10	10			
0.01	0	0	0	0	0	0			
0.005	0	0	0	0	0	0			
0.001	0	0	0	0	0	0			
Control	0	0	0	0	0	0			

One larva/cup; four replicates for each treatment rate and control.

ues of the tested repellents than the reported activity levels of two organophosphorus insecticides and an insect growth regulator (IGR) against some Copepoda and a cladoceran species suggest a several-fold safety margin of the experimental repellents to the aquatic invertebrates tested. For example, malathion and temephos were reported to have LC50 values of $<\!25$ ppb against species of Copepoda (Helgen et al. 1988, Naqvi and Hawkins 1989, Forget et al. 1998), and the IGR diflubenzuron had an LC50 value of 0.15 ppb against Daphnia magna (Julin and Sanders 1978).

The results of this study also indicate the severalfold higher LC50 values of the tested nontarget invertebrates when compared with laboratory and field LC_{50} values of 0.001-0.011% or 10-110 ppm required for anti-oviposition activity of Ae. albopictus with the three repellents (R.-D.X. and D.R.B., unpublished data). These repellents, even when used as larvicides for purposes of controlling younger instars of Ae. albopictus, may cause minimal or no adverse effects on the nontarget invertebrates because of the generally lower LC₅₀ values (0.0050 - 0.021% or 50 - 210 ppm) for first-instar Ae. albopictus (R.-D.X. and D.R.B., unpublished data) than for the invertebrates tested in the current study. However, for controlling fourth instars of Ae. albopictus (LC₅₀ = 0.019-0.034% or 190-340ppm) with the test repellents (R.-D.X. and D.R.B., unpublished data), the possibility exists that simultaneous reductions of chironomid larvae (LC₅₀ = 0.017-0.024 or 170-240 ppm) and first-instar Tx. amboinensis $(LC_{50} = 0.017-0.022\% \text{ or } 170-220 \text{ ppm})$ will occur because of the lower LC_{50} values of these nontarget organisms, compared with fourth-instar $Ae.\ albopictus.$ Nevertheless, within the context of using repellents as mosquito larvicides, the dosages required would be extremely high as compared with the activity of some mosquito larvicides and IGRs reported previously by Ali et al. (1995) where the LC_{50} values for $Ae.\ albopictus$ with some organophosphates ranged from 0.0033 to 0.379 ppm, with pyrethroids 0.00095 to 0.0052 ppm, and with IGRs 0.00011 to 0.0022 ppm.

Acknowledgments

We thank the following personnel of the CMAVE, USDA, Gainesville, FL: Jimmy Becnel and Peg Johnson for providing *Tx. amboinensis* larvae; Tok Fukuda and Tiny Willis for providing *S. spiculatus*; and Jean White for providing *E. agilis* and Joanna Taylor for technical help in the laboratory. The USDA-ARS, Insect Chemical Ecology Laboratory, Washington, DC, is acknowledged for providing experimental repellents AI3-37220 and AI3-35765. This is Florida Agricultural Experiment Station Journal Series No. R-06920.

References Cited

Ali, A., J. K. Nayar, and R. D. Xue. 1995. Comparative toxicity of selected larvicides and insect growth regulators to a Florida laboratory population of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 11: 72–76.

Dunkel, F. V., and D. C. Richards. 1998. Effect of an Azadirachtin formulation on six nontarget aquatic macroinvertebrates. Environ. Entomol. 27: 667–674.

Finney, D. J. 1971. Probit Analysis. Cambridge University Press, Cambridge, UK.

Forget, J., J. F. Pavillon, M. R. Menasria, and G. Bocquene. 1998. Mortality and LC₅₀ values for several stages of the marine copepod *Tigriopus brevicornis* (Müller) exposed to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos, and malathion. Ecotoxicol. Environ. Saf. 40: 239–244.

Helgen, J. C., N. J. Larson, and R. L. Anderson. 1988. Response of zooplankton and *Chaoborus* to temephos in a natural pond and in the laboratory. Arch. Environ. Contam. Toxicol. 17: 459–471.

Julin, A. M., and H. O. Sanders. 1978. Toxicity of the IGR diflubenzuron to freshwater invertebrates and fishes. Mosq. News 38: 256–259.

Mulla, M. S., and A. M. Khasawinah. 1969. Laboratory and field evaluation of larvicides against chironomid midges. J. Econ. Entomol. 62: 37–41.

Mulla, M. S., G. Majori, and A. A. Arata. 1979. Impact of biological and chemical mosquito control agents on nontarget biota in aquatic ecosystems. Residue Rev. 71: 121–173.

Naqvi, S. M., and R. H. Hawkins. 1989. Responses and LC_{50} values for selected micro-crustaceans exposed to Spartan, Malathion, Sonar, Weedtrine-D, and Oust pesticides. Bull. Environ. Contam. Toxicol. 43: 386–389.

Steel, R. D., and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed., McGraw-Hill, New York.

True Epistat Manual. 1989. True Epistat manual, 3rd ed. T. L. Gustafson [ed.]. Richardson, Texas.

Received for publication 26 May 1999; accepted 16 March 2000

 $^{{}^{}a}F = 0.44$; df = 0 2, 36; P > 0.05. ${}^{b}F = 2.23$; df = 1, 36; P > 0.05.

 $^{^{}c}F = 19.83$; df = 5, 36; P < 0.001.

 $^{^{}d}F = 9.13$; df = 1, 36; P < 0.001.